Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 7, No. 3, pp. 373-378, 1997

0960-894X/97 $17.00 + 0.00

PII: S0960-894X(97)00020-6

6-BROMO-3'-NITROFLAVONE, A NEW HIGH AFFINITY BENZODIAZEPINE RECEPTOR AGONIST RECOGNIZES TWO POPULATIONS OF CEREBRAL CORTICAL BINDING SITES

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Abstract: The synthesis and biochemical characterization of 6-bromo-3'-nitroflavone (**1**) is presented. Compound **1** has higher affinity for cerebellar and cerebral cortical than for striatal, hippocampal, or spinal cord benzodiazepine receptors (BDZ-Rs). In the cerebral cortex it recognizes two populations of binding sites (K_is 1.2 nM and 15.5 nM, respectively), and at doses of 0.01 to 0.3 mg/kg, ip produces anxiolytic effects in mice.

Introduction

We have recently found that some natural and synthetic flavonoids are selective and competitive ligands for the central BDZ-Rs with anxio-selective actions.[1-5] This led us to postulate that flavonoids represent a new family of BDZ-R ligands possessing pharmacological properties distinct from classical BDZs. Although most BDZs bind to the BDZ-Rs in every region of the brain with similar affinities, the pharmacological properties of several other BDZ-R ligands, most notably CL 218872, zolpidem, and some β-carbolines[6,7] have demonstrated the existence of at least two different types of BDZ-Rs in the CNS. Type I, found in most regions of the brain but predominantly in the cerebellum, is probably formed by the subunit combination $\alpha_1\beta_2\gamma_2$. Type II, found principally in the striatum, hippocampus, and spinal cord, is a mixture of subtypes containing α_2, α_3, and α_5 subunits.[8-11] The cerebral cortex has approximately equal proportions of both types.[11]

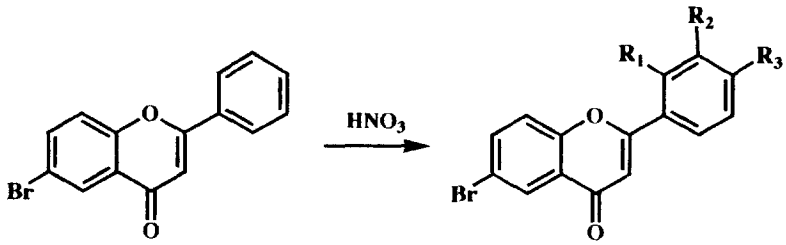
Here we report on the synthesis and biochemical characterization of 6-bromo-3'-nitroflavone (**1**), a new member of the flavonoid family of BDZ-R ligands that produces a potent anxiolytic action in mice and, most

significantly, exhibits a different affinity for BDZ-Rs types I and II. This property of **1** suggests its potential application to explore the pharmacological significance of different subtypes of BDZ-Rs.

Chemistry

6-bromo-3'-nitroflavone (**1**), 6-bromo-2'-nitroflavone (**2**), and 6-bromo-4'-nitroflavone (**3**) were prepared starting from 6-bromoflavone (**4**)[5] following Scheme 1 and an experimental procedure closely resembling that in reference 4. The eluates from the silica gel column were analyzed by TLC and accordingly, three homogeneous fractions were pooled, recrystalized, and used for identification rendering compounds **1**, **2**, and **3**.[12]

Scheme 1



4

1 $R_1 = H$ $R_2 = NO_2$ $R_3 = H$
2 $R_1 = NO_2$ $R_2 = H$ $R_3 = H$
3 $R_1 = H$ $R_2 = H$ $R_3 = NO_2$

Results and Discussion

Nonspecific nitration of compound **4** yielded three major products. Compounds **2** and **3** inhibited the binding of ^{3}H-flunitrazepam (^{3}H-FNZ) to extensively washed rat cerebral cortical membranes[13] with K_is of 208 ± 19 nM (n = 4) and 220 ± 1 nM (n = 2), respectively, but compound **1** was several times more active, as shown in Table 1 for various rat brain structures.

Table 1. Effect of compound **1** on ^{3}H-FNZ binding to synaptosomal membranes from different rat brain structures (see Figure 1).

STRUCTURE	n	$K_i \pm$ SEM (nM)
cerebellum	4	3.6 ± 0.1
hippocampus	3	9.6 ± 0.6
striatum	3	9.8 ± 1.6
spinal cord	3	12.7 ± 0.5
cerebral cortex	6	1.2 ± 0.4 and 15.5 ± 0.9

The potency of compound **1** in displacing ^{3}H-FNZ binding was highest in the cerebellum and in one population of cerebral cortical BDZ binding sites (see Figure 1 and Table 1). In the hippocampus, striatum, and spinal cord compound **1** exhibited a potency 3-4 times lower but in the spinal cord it had an affinity 10 times lower than the highest value found in the cerebral cortex (Table 1). In contrast, diazepam exhibits similar K_i values for displacement of ^{3}H-FNZ in all brain structures (app. 7 nM).

These results correlate well with the known distribution of BDZ-R subtypes in the brain and indicate a higher affinity of compound **1** for type I receptors since the cerebellum and the cortex are richer in this subtype while in the other structures shown in Table 1, type II predominates.[8-11]

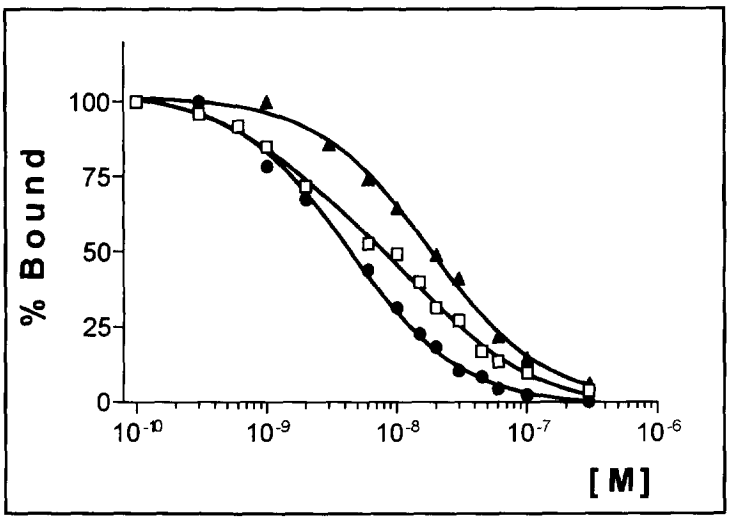


Figure 1: Binding of 0.5 nM ^{3}H-FNZ, to extensively washed crude synaptosomal membranes from rat cerebellum (●), cerebral cortex (□), and spinal cord (▲), was displaced by 9-14 different concentrations of compound **1.** Data are from a representative experiment replicated 3-6 times. The competition curves were analyzed using the Graph-Pad software (see results in Table 1).

The GABA-shift (ratio of K_i values of a competitive BDZ-R ligand in the presence or in the absence of GABA), is a routinely used biochemical index that predicts the behavioral effects and efficacy of drugs acting on these binding sites.[14] Displacements curves of ^{3}H-FNZ binding to cortical membranes, in the presence or absence of 100 μM GABA, showed that compound **1** behaves as a partial agonist (GABA-shift of compound **1** = 1.38 ± 0.10 (SEM), n = 3; experiments run in parallel with diazepam gave a GABA-shift of 2.00 ± 0.10 (SEM), n = 3).

Scatchard analysis of saturation curves of ^{3}H-FNZ binding to cerebral cortical membranes reveals that compound **1** is a competitive ligand for the BDZ-R (data not shown).

When a selective type I BDZ-R ligand like ^{3}H-zolpidem was used, compound **1** had similar K_i values for cerebellum and cerebral cortex (3 ± 1 nM and 3.8 ± 1 nM, n = 3, respectively). These affinity values are consistent with the nature of the cerebellar binding sites.[11]

Compound **1** appears to be a selective BDZ-R ligand because it does not displace (at 10 μM) ^{3}H-muscimol, ^{3}H-AMPA, ^{3}H-QNB, and ^{3}H-8-OH-DPAT binding for $GABA_A$, AMPA-glutamate, cholinergic-muscarinic, and serotonin 1_A receptors, respectively.

Preliminary pharmacological experiments in mice reveal that compound **1** (10-300 μg/kg, ip), has a potent anxiolytic effect in the elevated plus-maze,[15] increasing the percentage of entries in the open arms (10-100 μg/kg) and the time spent in these arms (100-300 μg/kg) (Figure 2). No changes were observed in the total arm entries. Experiments run in parallel with diazepam showed that it produces an increase in the percentage of open arm entries at a dose 10 times higher (vehicle = 22.9 ± 2.0%; diazepam 100 μg/kg = 35 ± 4.5 %, $p < 0.05$, n = 12).

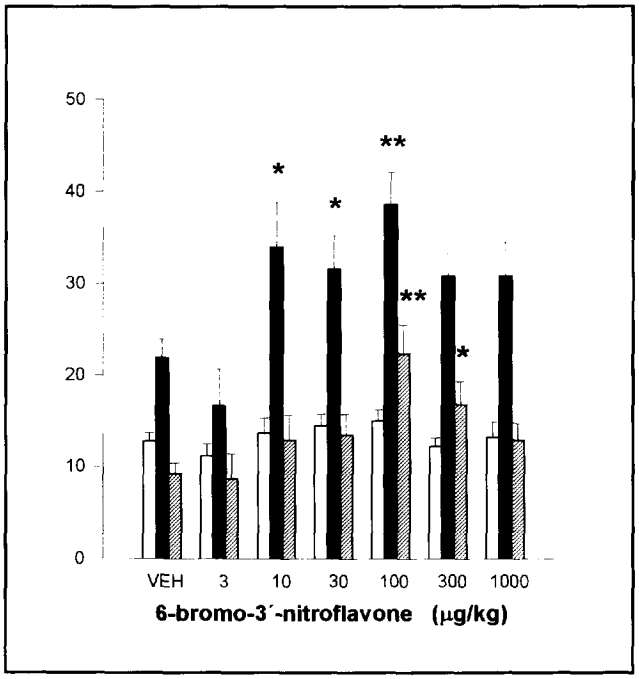


Figure 2: Mean ± SEM of total arms entries (open bars), percentage of open arms entries (closed bars) and percentage of time spent in the open arms (hatched bars) of mice given a 5 min session in the elevated plus-maze, 20 min after ip injection with vehicle (VEH) or compound **1** (3-1000 μg/kg). *$p < 0.05$, **$p < 0.01$, significantly different from vehicle (Dunnett multiple comparison test after ANOVA). Number of animals in the experimental groups ranged between 13 and 28.

In conclusion, we have presented evidence supporting the assumption that compound **1** is a high affinity BDZ-R ligand with agonistic properties, which recognizes two populations of binding sites in the cerebral cortex and displays a differential potency for the inhibition of ^{3}H-FNZ binding in several regions of the rat brain, in accordance with the regional distribution of type I BDZ-R. Compound **1** is 10 times more potent as anxiolytic than diazepam, as evidenced in the elevated plus-maze test.

The present results further endorse our hypothesis that natural and synthetic flavonoids represent a new family of BDZ-R ligands with interesting biochemical and pharmacological profiles.[16]

Acknowledgements: This work has been supported by grants from the National Research Council of Argentina (CONICET), the University of Buenos Aires, OAS (Washington, USA) and the Strathclyde Institute for Drug Research of the University of Strathclyde, Glasgow, UK.

References and Notes

1. Medina, J. H.; Paladini, A. C.; Levi de Stein, M.; Calvo, D.; Diaz, L. E.; Peña, C. *Biochem. Pharmacol.* **1990**, *40*, 2227.

2. Wolfman, C.; Viola, H.; Paladini, A. C.; Dajas, F; Medina, J. H. *Pharmacol. Biochem. Behav.* **1994**, *47*, 1.

3. Viola, H.; Wasowski,C.; Levi de Stein, M.; Wolfman, C.; Silveira, R; Dajas, F.; Medina, J. H.; Paladini, A. C. *Planta Medica* **1995**, *61*, 213.

4. Marder, M.; Viola, H.; Wasowski, C.; Wolfman, C.; Waterman, P.; Medina, J. H.; Paladini, A. C. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2717.

5. Marder, M.; Viola, H.; Wasowski, C.; Wolfman, C.; Waterman, P.; Cassels, B. K.; Medina, J. H.; Paladini, A. C. *Biochem. Biophys. Res. Commun.* **1996**, *223*, 384.

6. Klepner, C. A.; Lippa, A. S.; Benson, D. I.; Sano, M. C.; Beer, B. *Pharmacol. Biochem. Behav.* **1979**, *11*, 457.

7. Arbilla, S.; Allen, J.; Wick, A.; Langer, S. Z. *Eur. J. Pharmacol.* **1986**, *130*, 257.

8. Sieghart, W. *Trends Pharmacol. Sci.* **1992**, *13*, 446.

9. Doble, A.; Martin, I. L. *Trends Pharmacol. Sci.* **1992**, *13*, 76.

10. Wisden, W.; Laurie, D. J.; Monyer, H.; Seeburg, P. *J. Neurosci.* **1992**, *12*, 1040.

11. McKernan, R. M.; Whiting, P. J. *Trends Neurosci.* **1996**, *9*, 139.

12. The three compounds gave MS and elemental analytical data consistent with their structures.

Compound **1**: yield 40%; light yellow crystals (from acetone-water); ^{1}H NMR (300 Mhz, $CDCl_3$) δ 8.80 (t, J = 2.0 Hz, H-2'), 8.42 (dt, J = 8.0, 2.4 Hz, H-4'), 8.37 (d, J = 2.4 Hz, H-5), 8.21 (dt, J = 8.0, 2.0 Hz, H-6'), 7.84 (dd, J = 8.8, 2.5 Hz, H-7), 7.76 (t, J = 8.2 Hz, H-5'), 7.54 (d, J = 9.2 Hz, H-8), 6.92 (s, H-3).

Compound **2**: yield 20%; yellow crystals (from acetone-water); ^{1}H NMR (300 Mhz, $CDCl_3$) δ 8.37-8.42 (m, H-5, H-2', H-6'), 8.10 (d, J = 8.8 Hz, H-3', H-5'), 7.84 (dd, J = 9.0, 2.4 Hz, H-7), 7.52 (d, J = 9.0 Hz, H-8), 6.92 (s, H-3).

Compound **3**: yield 40%; light yellow crystals (from acetone-water); ^{1}H NMR (300 Mhz, $CDCl_3$) δ 8.37 (d, J = 2.4 Hz, H-5), 8.11 (dd, J = 1.5, 7.7 Hz, H-6'), 7.75 (m, H-7, H-3', H-4', H-5'), 7.29 (d, J = 9.0 Hz, H-8), 6.60 (s, H-3).

13. The preparation of the synaptosomal membranes from different brain structures and the binding of ^{3}H-FNZ (81.8 Ci/mmol; NEN) were carried out as described by Levi de Stein, M.; Medina, J. H.; De Robertis, E. *Mol. Brain Res.* **1985**, *5*, 9.

14. Ehlert, F. J; Roeske, W. R; Gee, K. W; Yamamura, H. I. *Biochem. Pharmacol.* **1983**, *32*, 2375.

15. The animals and the pharmacological test used were as described in reference 4.

16. Medina, J. H.; Viola, H.; Wolfman, C.; Marder, M.; Wasowski, C.; Calvo, D.; Paladini, A. C. *Neurochem. Res.* **1996**, in press.

(*Received in USA* 9 *September* 1996; *accepted* 6 *January* 1997)